LOCAL FACTORS IN THE MECHANISM OF CALCIFICATION

by

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The composition of new mineral deposited when rachitic tibiae are calcified *in vitro* is related to fluid composition. The PO_4:CO_3, the Ca:P, and the citrate:PO_4 of the solid can be expressed as the logarithmic functions of the ratios of these same ions in the fluid. In growing Wistar and Cotton rats the PO_4:CO_3 of the bone, enamel, and dentin of growing molars as well as incisors, are related to the PO_4:CO_3 of blood serum. The PO_4:CO_3 of blood serum is regulated by the dietary PO_4:Ca. The X-ray diffraction pattern of the mineral deposited both in *in vivo* and *in vitro* calcification is characteristic of apatite.

These studies indicate that while the "local factor" of calcification may determine the site of mineralization, the composition of the mineral deposited is influenced by the composition of the fluid from which these precipitates form, and that such minerals of widely varying composition both in new and old calcification, are predominantly apatites.

In view of the preferential solubility of carbonate in bone and tooth mineral, caries susceptibility studies were undertaken. Studies to date, carried out in conjunction with Professor James H. Shaw of the Harvard School of Dental Medicine, indicate that Cotton Rats with high carbonate teeth are decisively more caries susceptible than litter mate animals with low carbonate teeth.

Earlier studies indicate that phosphatase and the enzymes involved in phosphorylative glycogenolysis are involved in the calcifying process. However, phosphatase can be inactivated with heat, silver, and mercury ions, yet calcification *in vitro* can take place with inorganic solutions. Calcification *in vitro* can be inactivated with strontium ions without inactivating phosphatase. For this reason the possible role of other factors, more particularly that of chondroitin sulfate, was investigated to explain some of the earlier findings. For example,

the disappearance of the calcifying mechanism in a basal salt solution is markedly retarded by calcium and strontium ions. Moreover, the calcifying mechanism can be reactivated by calcium ions following inactivation by beryllium, copper, magnesium, sodium and strontium ions, in which case the inactivation is a function of the ratio of inactivator to calcium ions. These results appear to indicate that combination of calcium ions with some local factor(s), possibly chondroitin sulfate, is an essential preliminary step to mineralization.

Heated bone sections, later treated with calcium ions, still retain their ability to calcify. While the heating process destroys enzymes in the phosphorylative cycle, chondroitin sulfate remains, as shown by metachromatic staining. Further investigations were undertaken, therefore, to clarify the role of this metachromatic staining substance (presumably chondroitin sulfate) in the calcifying mechanism of preosseous cartilage. Inactivation with protamine and toluidine blue(compounds which combine avidly with sulfated mucopolysaccharides) is a function of the inactivator to calcium ratio. From these studies one might postulate that chondroitin sulfate is an integral part of the "local factor" responsible for calcification.

In attempting to relate the "local factor" to the state of polymerization of chondroitin sulfate, the influence of calcium ions(which reactivate the calcifying mechanism) on metachromatic staining was investigated. It was shown that with a constant amount of toluidine blue the degree of metachromasia increases with the calcium ion concentration in solution, reaching a maximum at about 15 milliequivalents of calcium ion per liter. A further increase of calcium ion causes a gradual decrease in the degree of metachromasia. It was also shown that prior shaking with calcium chloride enhances the intensity of metachromatic staining in the ossifying cartilage matrix. Such staining is not pre-

vented by simple soaking with distilled water or alcohol, as in the case of metachromatic staining in the absence of prior treatment with calcium ions. In contrast to this when chondroitin sulfate is extracted from rachitic cartilage, calcium ion competitively interferes with metachromatic staining. These findings indicate the existence of some complex of chondroitin sulfate in the bone cartilage, which behaves differently with respect to calcium ion than chondroitin sulfate alone.

The appearance of metachromatic staining in bone cartilage did not correlate in all cases with calcifiability. However, the property of calcium ions increasing the degree of metachromasia seems to be typical of calcifying cartilage in the studies carried out to date. When calcifiability was destroyed by various agents metachromasia, if not also destroyed, was not enhanced by calcium ions.

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